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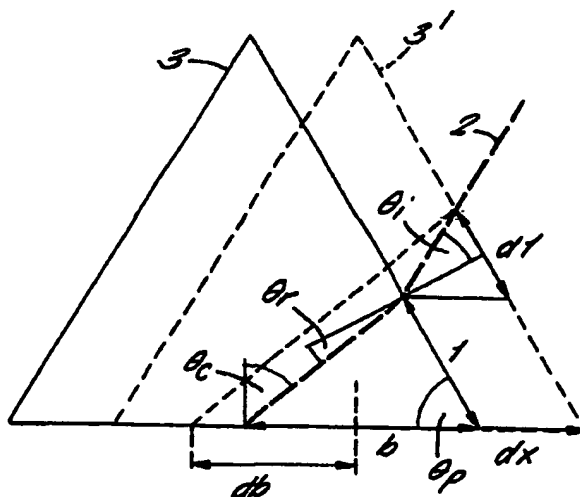
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- (71) Applicant (for all designated States except US): SOLEXA LTD. [GB/GB]; Chesterford Research Park, Little Chesterford, Nr. Saffron Walden, Essex CB10 1XL (GB).
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- (72) Inventors; and
- (75) Inventors/Applicants (for US only): OSBORNE, Mark [GB/GB]; Flat 14, 50 Dyke Road, Brighton BN1 3JB (GB). BRYANT, Jason [GB/GB]; 25 Watermead, Bar Hill, Cambridge CB3 8TJ (GB).
- (74) Agents: CROSS, Rupert, E., B., et al.; BOULT WADE TENNANT, Verulam Gardens, 70 Grays Inn Road, London WC1X 8BT (GB).
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(54) Title: PRISM DESIGN FOR OPTICAL SCANNING APPLICATIONS



(57) Abstract: There is disclosed a prism for use in scanning applications such as total internal reflection microscopy in which the prism is translated relative to an incident light beam. A geometry is disclosed which cancels walk of the beam footprint at the base of the prism.

## PRISM DESIGN FOR OPTICAL SCANNING APPLICATIONS

The present invention relates to scanning applications in which a sample, together with an angular optic ie a triangular or trapezoidal prism, are scanned in a fixed laboratory frame of reference. The fixed laboratory frame is defined by a fixed light source generating a fixed light beam and a detector. The angular optic couples the light beam to the base interface of the angular optic where the sample is located, such that the beam is incident on the base interface at an off normal angle. In such applications it is desirable that the intercept of the light beam at the base interface of the angular optic (the "footprint") remains stationary in the laboratory frame as the optic and sample are scanned so that there is no loss of image integrity at the detector.

One example of such an application is Total Internal Reflection Microscopy, which is a technique for observing samples illuminated by an evanescent wave. Total internal reflection occurs when a beam of light travelling through a very dense medium such as glass encounters an interface with a less dense medium such as air or water, at an angle to the normal which is greater than the critical angle for the interface. The critical angle for a glass/water interface is given by Fresnel's Law of Refraction as:

$$\theta_c = \sin^{-1}(n_{\text{water}}/n_{\text{glass}})$$

At angles greater than the critical angle, when total internal reflection takes place, an electric field component of the light penetrates through the interface into the water as an evanescent wave. The evanescent wave has the same wavelength as the incident beam but penetrates only a very short distance into the water, typically no more than 1  $\mu\text{m}$ . The evanescent wave decays exponentially from the interface into the water with a characteristic

penetration depth dependent on the wavelength and angle of incidence of the totally internally reflected light.

In Total Internal Reflection Fluorescence Microscopy, fluorophores may be excited by the light in the evanescent field if they are close to the glass/water interface, but fluorophores further away in the bulk of the solution will not be excited. The result is that images with very low background fluorescence are obtained. Figure 1 shows a typical instrument set up used in Total Internal Reflection Fluorescence Microscopy. A sample is placed such that it is located directly on the interface of the base of a light coupling optic or dispersion prism. Alternatively, a glass slide may be optically matched to the prism, and the sample located on the base of the slide. Total internal reflection then occurs at the base of the slide. Typically, the objective lens and external light source are fixed in the lab frame and the sample on which the light coupling optic or prism is fixed is scanned in a plane perpendicular to the objective lens axis. The prism therefore moves relative to the objective lens and the light source. Conventionally a 45° or 60° dispersion prism is used, but to obtain light beams incident on the base of the prism at angles close to and greater than the critical angle, the light must usually be incident on the input face of the prism at off normal angles of incidence to achieve refraction of the beam at the air/glass interface. The deviation of the beam causes the reflection footprint at the base of the prism to walk  $db$  as the prism is translated  $dx$  towards or away from the light source. In a limiting case light propagating parallel to the prism base will be refracted such that the footprint at the prism base moves equally and in the same direction as the prism ( $db/dx = 0$ ). In this case the footprint moves  $dx$  in the lab frame and the illuminated area moves rapidly away from the imaging lens as the sample is scanned.

It is an object of the present invention to obtain a footprint which is static in the laboratory frame of reference, defined by the objective lens and light source, such that the area illuminated at the glass/aqueous interface does not move away from the optical axis of the lens as the sample and prism are scanned.

According to the present invention, a scanning apparatus comprises:-

a light source for generating a light beam; and

an angular prism coupled to a sample at a base interface;

characterised in that the base angle  $\theta_p$  of the prism satisfies the equations:-

$$\frac{(\cos^2\theta_c - \sin^2\theta_p)(\tan\theta_i \sin\theta_p + \cos\theta_p)}{\cos\theta_c \cos(\theta_p \pm \theta_c)} = 1$$

and

$$n_i \sin\theta_i = n_p \sin(\theta_p - \theta_c)$$

wherein  $\theta_c$  is the coupling angle required for light incident at the base interface of the prism,  $\theta_i$  is the incident angle of the light beam on the prism,  $n_i$  is the refractive index of the medium at the interface where the light beam enters the prism and  $n_p$  is the refractive index of the prism.

According to the present invention, a scanning method comprises the steps of:-

generating a light beam;

providing an angular prism in the path of the light beam, the prism being coupled to a sample at a base interface; and

5 moving the prism and sample relative to the light beam;

characterised in that the base angle  $\theta_p$  of the prism satisfies the equations:-

10

$$\frac{(\cos^2\theta_c - \sin^2\theta_p) (\tan\theta_i \sin\theta_p + \cos\theta_p)}{\cos\theta_c \cos(\theta_p +/\!-\theta_c)} = 1$$

and

15

$$n_i \sin\theta_i = n_p \sin(\theta_p - \theta_c)$$

wherein  $\theta_c$  is the coupling angle required for light incident at the interface at the base interface of the prism,  $\theta_i$  is the incident angle of the light beam on the prism,  $n_i$  is the refractive index of the medium at the interface where the light beam enters the prism and  $n_p$  is the refractive index of the prism.

25 It has been found that, if the prism satisfies the above criteria, a solution exists where the footprint of the light beam on the base of the prism walks in an equal and opposite direction to the prism's translation in the lab frame i.e.  $db = -dx$ . Therefore, the point where the light incident to the prism intercepts the prism base is fixed in the lab frame. This results in scanning of the sample without movement of the footprint with respect to a detector fixed in the lab frame and hence no loss of image integrity.

35

A solution is found wherein  $\theta_p = \theta_c$  and  $\theta_i = 0$ .

Preferably, the apparatus comprises a total internal reflection microscopy apparatus and includes means for detecting interaction of the sample at the base of the prism or optically matched slide with an evanescent wave  
5 formed by total internal reflection of the light beam at the base of the prism or at the base of a slide which is optically matched to the prism. Preferably, the method according to the present invention includes the step of detecting interaction of a sample at the base of the prism  
10 with an evanescent wave formed by total internal reflection of the light beam at the base of the prism.

For light entering the prism from air,  $n_1 = 1$ , and  
15 therefore

$$\sin \theta_i = n_p \sin (\theta_p - \theta_c).$$

For total internal reflection to occur at the base  
20 interface  $\theta_c$  must be greater than or equal to the critical angle for the interface ie

$$\theta_c \geq \sin^{-1} (n_s/n_p)$$

25 Wherein  $n_s$  is the refractive index of the sample medium.

In total internal reflection microscopy, it is preferable that the coupling angle is greater than but  
30 close to the critical angle as this maximises the penetration of the evanescent wave into the sample medium. It is generally preferable that  $\theta_c$  is slightly above the critical angle because, although penetration of the evanescent wave is at a maximum at the critical angle,  
35 there will be a spread of angles within the beam and, to ensure total internal reflection of the entire beam it is preferable to have  $\theta_c$  slightly above the critical angle.

This also accommodates minor variations in the refractive indices of the interfacial media.

For a quartz/water interface at the base of the prism,  
5 where  $n_p=1.46$ , the critical angle of the base interface is  $66^\circ$ . A preferred value for  $\theta_c$  would be  $68^\circ$ . A unique solution is found wherein  $\theta_i = 0$ , and  $\theta_p = 68^\circ$ .

A preferred embodiment of the present invention will  
10 now be described with reference to the accompanying drawings; in which:-

Figure 1 shows an apparatus for Total Internal  
Reflection Fluorescence Microscopy;

15

Figure 2 is a schematic showing the total internal reflection footprint walking with the displacement of the prism; and,

20 Figure 3 is a graph of  $db/dx$  against prism angle for a quartz/water base interface and a coupling angle of  $68^\circ$ .

Figure 1 illustrates an apparatus for Total Internal Reflection Fluorescence Microscopy having a light source 1  
25 which generates a beam 2 which is incident on a prism 3 and is totally internally reflected at the base interface of the prism 3. A sample is positioned adjacent to a base interface of the prism 3 and the evanescent wave interacts with the sample, producing fluorescence. The fluorescence  
30 passes through an objective lens 4 and is directed towards a CCD camera 5 by a mirror 6, passing through a filter 7.

As shown in Figure 2, the light beam 2 is incident on the prism 3 at  $\theta_i$  to the normal, is refracted as it enters  
35 the prism at  $\theta_r$  to the normal and is incident on the base of the prism 3 at a coupling angle  $\theta_c$ , forming a footprint at the base interface. For total internal reflection to

occur,  $\theta_c$  must be at least the critical angle for the base interface. As the prism moves  $dx$  in the lab frame to position 3', the footprint walks  $db$  in the prism frame.

5 The magnitude of the differential  $dl/db$  may be derived as follows:-

Sine rule  $\frac{l}{\sin(90-\theta_c)} = \frac{r}{\sin\theta_p}$  or  $r \cos\theta_c = l \sin\theta_p$  (1)

10 Cosine rule  $r^2 = b^2 + l^2 - 2bl \cos\theta_p$  (2)

(1) in (2)  $l^2 \sin^2\theta_p = \cos^2\theta_c (b^2 + l^2 - 2bl \cos\theta_p)$   
 15 quadratic in  $l$   $l^2 (\cos^2\theta_c - \sin^2\theta_p) - 2bl \cos\theta_p \cos^2\theta_c + b^2 \cos^2\theta_c = 0$

20 solution to which is

$$l = \frac{2b \cos\theta_p \cos^2\theta_c + \sqrt{4b^2 \cos^4\theta_c \sin^2\theta_p - 4b^2 \cos^2\theta_c (\cos^2\theta_c - \sin^2\theta_p)}}{2(\cos^2\theta_c - \sin^2\theta_p)}$$

expand contents of the square root

25  $l = \frac{2b \cos\theta_p \cos^2\theta_c + \sqrt{4b^2 \cos^4\theta_c \sin^2\theta_p - 4b^2 \cos^4\theta_c + 4b^2 \cos^2\theta_c \sin^2\theta_p}}{2(\cos^2\theta_c - \sin^2\theta_p)}$

take out  $4b^2 \cos^2\theta_c$  common in the square root

30  $l = \frac{2b \cos\theta_p \cos^2\theta_c + 2b \cos\theta_c \sqrt{\cos^2\theta_c \sin^2\theta_p - \cos^2\theta_c + \sin^2\theta_p}}{2(\cos^2\theta_c - \sin^2\theta_p)}$

factorizing gives

35  $l = \frac{b \cos\theta_c (\cos\theta_p \cos\theta_c + \sqrt{\cos^2\theta_c \cos^2\theta_p - \cos^2\theta_c + \sin^2\theta_p})}{(\cos^2\theta_c - \sin^2\theta_p)}$

differentiating w.r.t.  $b$

40  $\frac{dl}{db} = \frac{\cos\theta_c (\cos\theta_p \cos\theta_c + \sqrt{\cos^2\theta_c \cos^2\theta_p - \cos^2\theta_c + \sin^2\theta_p})}{(\cos^2\theta_c - \sin^2\theta_p)}$

further factorization gives

45  $\frac{dl}{db} = \frac{\cos\theta_c (\cos\theta_p \cos\theta_c + \sqrt{\cos^2\theta_c (\cos^2\theta_p - 1) + \sin^2\theta_p})}{(\cos^2\theta_c - \sin^2\theta_p)}$



recognising  $\cos^2\theta_p - 1 = -\sin^2\theta_p$  and factorising

$$5 \quad \frac{dl}{db} = \frac{\cos\theta_c(\cos\theta_p\cos\theta_c + \sqrt{\sin^2\theta_p(1-\cos^2\theta_c)})}{(\cos^2\theta_c - \sin^2\theta_p)}$$

recognising  $1-\cos^2\theta_c = \sin^2\theta_c$  and rooting the square

$$10 \quad \frac{dl}{db} = \frac{\cos\theta_c(\cos\theta_p\cos\theta_c + \sin\theta_p\sin\theta_c)}{(\cos^2\theta_c - \sin^2\theta_p)}$$

using trigonometric identity the differential simplifies to

$$15 \quad \frac{dl}{db} = \frac{\cos\theta_c\cos(\theta_p + \theta_c)}{(\cos^2\theta_c - \sin^2\theta_p)}$$

The magnitude of the differential  $dl/dx$  may be derived as follows:-

20

$$\text{Sine rule} \quad \frac{dl}{\sin(90-\theta_p+\theta_1)} = \frac{dx}{\sin(180-\theta_p-(90-\theta_p+\theta_1))}$$

25

$$\text{Simplifies to} \quad dl \sin(90-\theta_1) = dx \sin(90-\theta_p+\theta_1)$$

$$dl \cos\theta_1 = dx \cos(\theta_p - \theta_1)$$

30

$$\frac{dl}{dx} = \frac{\cos\theta_p\cos\theta_1 + \sin\theta_p\sin\theta_1}{\cos\theta_1}$$

$$\frac{dl}{dx} = \cos\theta_p + \tan\theta_1\sin\theta_p$$

35 Recognising that a translation of the prism  $dx$  in the positive  $x$  direction results in a displacement  $db$  of the footprint in the negative direction and by using the chain rule

$$-\frac{db}{dx} = \frac{dl}{dx} \times \frac{db}{dl}$$

40 so

$$\frac{db}{dx} = \frac{-(\cos\theta_p + \tan\theta_1\sin\theta_p) \times (\cos^2\theta_c - \sin^2\theta_p)}{\cos\theta_c\cos(\theta_p \pm \theta_c)}$$

where  $\theta_i = \sin^{-1}(n_p \sin(\theta_p - \theta_c))$  from Fresnel's equation, for light entering the prism from air, ( $n_1 = 1$ ).

5 For the footprint to remain stationary in the lab frame, it must walk in an equal and opposite direction in the prism frame to the prism's translation in the lab frame, i.e.  $db/dx = -1$ .

10 In total internal reflection microscopy, it is preferred that the coupling angle is greater than but close to the critical angle as this maximises the penetration of the evanescent wave into the sample medium, and it is generally preferable that  $\theta_c$  is slightly above the critical angle because, although penetration of the evanescent wave  
15 is at a maximum at the critical angle, there will be a spread of angles within the beam and, to ensure total internal reflection of the entire beam, it is preferable to have  $\theta_c$  slightly above the critical angle.

20 For a quartz/water interface at the base of the prism, where  $n_p=1.46$ , the critical angle of the base interface is  $66^\circ$ . A preferred value for  $\theta_c$  would be  $68^\circ$ . Figure 3 shows the degree of walking of the footprint with prism displacement as a function of the internal angle  $\theta_p$  of the  
25 prism for  $\theta_c = 68^\circ$ . The prism angle required for  $db/dx = -1$  is  $68^\circ$  and the light will be incident normal to the input surface of the prism, i.e.  $\theta_i = 0$ .

CLAIMS

1. A scanning apparatus comprising:-

5 a light source for generating a light beam; and

an angular prism coupled to a sample at a base interface;

10 characterised in that the base angle  $\theta_p$  of the prism satisfies the equations:-

$$\frac{(\cos^2\theta_c - \sin^2\theta_p)(\tan\theta_i \sin\theta_p + \cos\theta_p)}{\cos\theta_c \cos(\theta_p +/\theta_c)} = 1$$

15

and

$$n_i \sin\theta_i = n_p \sin(\theta_p - \theta_c)$$

20 wherein  $\theta_c$  is the coupling angle required for light incident at base interface of the prism,  $\theta_i$  is the incident angle of the light beam on the prism,  $n_i$  is the refractive index of the medium at the interface where the light beam enters the prism and  $n_p$  is the refractive index of the prism.

25

2. A scanning apparatus according to claim 1, wherein  $\theta_p = \theta_c$  and  $\theta_i = 0$ .

30 3. A total internal reflection microscopy apparatus according to claim 1 or 2, including means for detecting interaction of the sample at the base of the prism with an evanescent wave formed by total internal reflection of the light beam at the base of the prism.

35

4. A total internal reflection microscopy apparatus according to claim 3, wherein

$$\theta_c \geq \sin^{-1} (n_s/n_p)$$

and wherein  $n_s$  is the refractive index of the sample medium.

5

5. A scanning method comprising the steps of:-

generating a light beam;

10 providing an angular prism in the path of the light beam, the prism being coupled to a sample at a base interface; and

15 moving the prism and sample relative to the light beam;

characterised in that the base angle  $\theta_p$  of the prism satisfies the equations:-

$$20 \quad \frac{(\cos^2 \theta_c - \sin^2 \theta_p) (\tan \theta_p \sin \theta_c + \cos \theta_p)}{\cos \theta_c \cos (\theta_p \pm \theta_c)} = 1$$

and

$$25 \quad n_i \sin \theta_i = n_p \sin (\theta_p - \theta_c)$$

30 wherein  $\theta_c$  is the coupling angle required for light incident at the interface at the base interface of the prism,  $\theta_i$  is the incident angle of the light beam on the prism,  $n_i$  is the refractive index of the medium at the interface where the light beam enters the prism and  $n_p$  is the refractive index of the prism.

35 6. A method of total internal reflection microscopy according to claim 5, further comprising the step of detecting interaction of a sample at the base of the prism with an evanescent wave formed by total internal reflection of the light beam at the base of the prism.

FIG. 1.

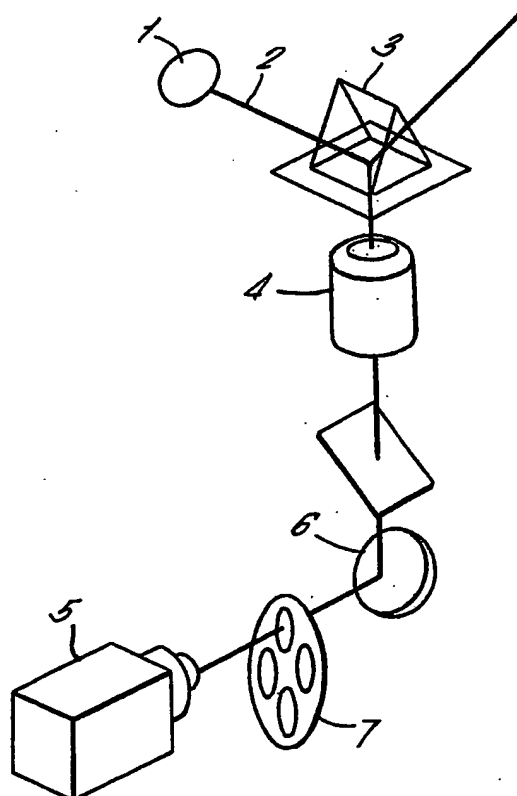


FIG. 2.

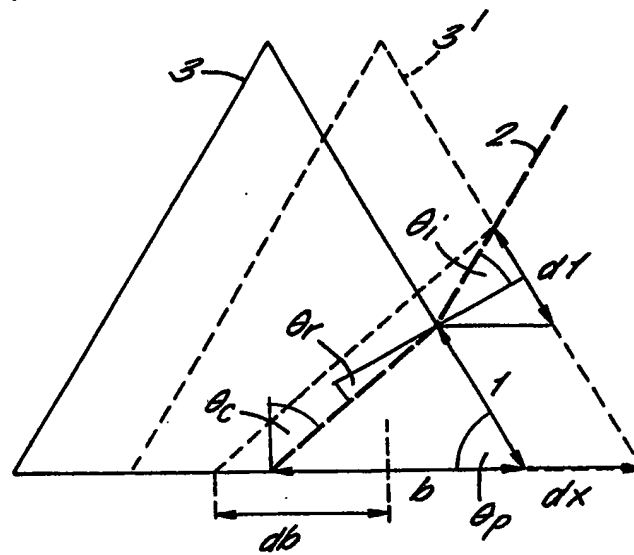
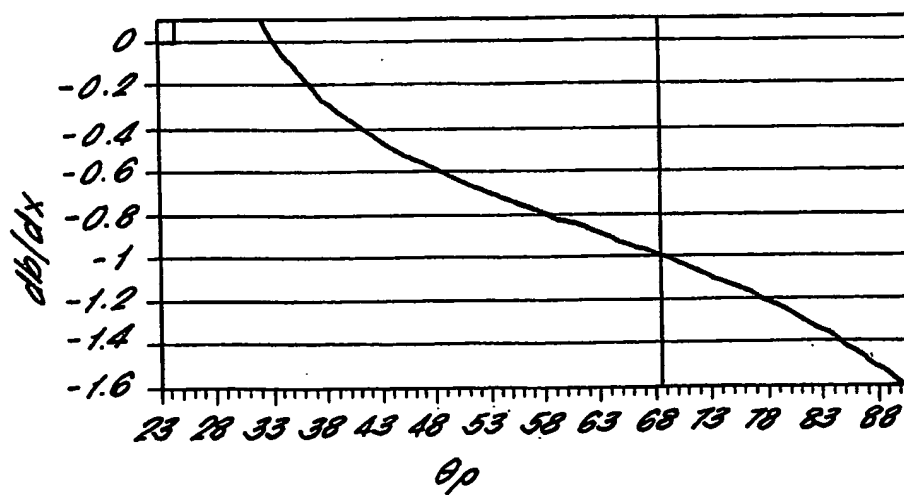


FIG. 3.



# INTERNATIONAL SEARCH REPORT

PC T/GB 03/00154

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC 7 G02B21/00 G02B5/04		
According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) IPC 7 G02B G01N		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, WPI Data, PAJ		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6 327 238 B1 (NISHIWAKI SEIJI ET AL) 4 December 2001 (2001-12-04) abstract; figures 2-5 column 10, line 9 - line 27	1-6
A	US 5 980 831 A (STONE JAMES J ET AL) 9 November 1999 (1999-11-09) the whole document	1-6
A	US 6 144 498 A (BRYARS BRETT J ET AL) 7 November 2000 (2000-11-07) example 1; table 1	1-6
<input type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 18 March 2003		Date of mailing of the international search report 26/03/2003
Name and mailing address of the ISA European Patent Office, P.B. 6818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fac (+31-70) 340-3016		Authorized officer Michel, A

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